Int J Clin Exp Pathol 2015;8(4):3971-3978
www.ijcep.com /ISSN:1936-2625/IJCEP0006349

Original Article

Effects of Smad decoy ODN on shear stress-induced atherosclerotic ApoE-/-mouse

Hyun-Jin An[1], Woo-Ram Lee[1], Kyung-Hyun Kim[1], Jung-Yeon Kim[1], Woon-Hae Kim[1], Kwan-Kyu Park[1], Sung Won Youn[2]

[1]*Department of Pathology, College of Medicine, Catholic University of Daegu, Daegu, South Korea;* [2]*Department of Radiology, College of Medicine, Catholic University of Daegu, Daegu, South Korea*

Received January 26, 2015; Accepted March 23, 2015; Epub April 1, 2015; Published April 15, 2015

Abstract: Atherosclerosis is a complex disease which involves both genetic and environmental factors in its development and progression. Shear stress is the drag force per unit area acting on the endothelium as a result of blood flow, and it plays a critical role in plaque location and progression. TGF-β1 is often regarded to have pro-atherosclerotic effect on vascular disease. TGF-β1 downstream targets Smad, for regulating a set of genes associated with atherosclerosis. Therefore, modulation of TGF-β1 and Smad expression may be the important targets for the prevention and treatment of shear stress-induced vascular disease. However, the precise mechanism of the anti-atherosclerotic effects of novel therapeutic approach has not been elucidated by using animal models regarding the shear stress-induced vascular disease. Therefore, we designed to test whether Smad decoy ODN would prevent the development of atherosclerosis in the shear stress-induced ApoE-/-mice on a western diet. We examined the effect of cast placement on the development of atherosclerosis, and the carotid artery was harvested at the sacrifice to observe histological changes. Also, we evaluated the impact of Smad decoy ODN in the regulation of genes expression related to atherosclerosis, including TGF-β1, PAI-1, and α-SMA. Our results showed that western diet with cast placement developed atherosclerosis in ApoE-/-mouse. Also, administration of Smad decoy ODN decreases the expression of TGF-β1, PAI-1, and α-SMA. These results demonstrate the potential of Smad decoy ODN to prevent the progression of atherosclerosis in ApoE-/-mouse model with western diet and shear stress.

Keywords: Atherosclerosis, shear stress, TGF-β1, Smad, decoy

Introduction

Atherosclerosis is a complex disease which involves both genetic and environmental factors in its development and progression. Its various risk factors include hypertension, obesity, diabetes, smoking and high fat contents in the daily diet [1, 2]. Atherosclerotic lesions commonly occur at the outer walls of arterial branches, and the inner curvatures of tortuous vessels. At these sites, the local flow is disturbed and it is characterized by low shear stress recirculation, oscillation, or lateral flow [3]. These shear stress is the drag force per unit area acting on the endothelium as a result of blood flow, and it plays a critical role in plaque location and progression [4, 5]. In straight arteries, laminar flow with an average shear stress of 1.5 N/m^2 prevails, and this level is actively maintained by adjusting the vascular tone and by structural remodeling in response to shear stress [6, 7]. In addition, low shear stress (< 1.5 N/m^2) and oscillatory shear stress (exhibiting directional change) have been implicated as pro-atherogenic in the observational studies for humans and animals [4, 8, 9]. Consequently, these shear stresses are critical in regulating the vascular physiology and pathology of the vessel wall [10, 11].

The pathogenic features of atherosclerosis show that it is a multi-factorial, progressive disease in which the inflammatory reaction of vessel wall and the inflammation-related factors play important roles at all stages [12, 13]. TGF-β1 is often regarded as having pro-atherosclerotic effect on vascular disease [14]. TGF-β1 facilitates extracellular matrix deposition by stimulating the production of pro-collagen and fibronectin, down-regulating the expression of proteases, and up-regulating protease inhibitors, such as plasminogen activator inhibitor

type I (PAI-I) and tissue inhibitor of metalloproteinase-1 (TIMP-1) [15-17]. TGF-β1 transgene into vascular wall causes intimal thickening in the presence or absence of vascular injuries in an animal models [18, 19]. Also, TGF-β1 downstream targets Smad, for regulating a set of genes associated with atherosclerosis [20, 21]. Therefore, modulation of TGF-β1 and Smad expression may be important targets for the prevention and treatment of shear stress-induced vascular disease. How-ever, the relation between shear stress and atherosclerosis is based almost exclusively on clinical observations in humans or in vitro experiments [8, 9, 11, 22]. Recently, Chen et al. have introduced a new technique, which allows for the controlled study regarding the influence of wall shear stress on the development of atherosclerosis [4]. The method involves an innovative tapered restriction (cast) surgically placed around the right common carotid artery of an ApoE-/- mouse fed with lipid-rich diet. However, the precise mechanism of the anti-atherosclerotic effects of novel therapeutic approach has not been elucidated in animal models with shear stress-induced vascular disease.

To develop a novel therapeutic approach, we modified decoy oligodeoxynucleotide (ODN) against transcription factor Smad, into a ring-type structure without chemical modification, to increase its resistance to endonuclease for systemic administration. The decoy ODN, which contains a consensus sequence that binds to the target transcription factor, blocks mRNA transcription at the DNA level. The decoy approach is a new class of antigene strategy that utilizes modulation of endogenous transcriptional regulation [23]. The decoy is a synthetic double-stranded cis-element ODN, and chemical modifications such as phosphorothioation are usually utilized to increase the stability [24]. Therefore, we designed to test whether Smad decoy ODN would prevent the development of atherosclerosis in shear stress-induced ApoE-/-mouse on a western diet. Subsequently, we investigated anti-atherosclerotic effects of Smad decoy ODN, which blocks the TGF-β1 and extracellular matrix deposition.

Materials and methods

Experimental model

Eight-weeks-old ApoE-/-mice in a C57BL/6 background were obtained from Taconic (NY, USA). Mice were maintained in a room set at 21-25°C with a 12 hours light/dark cycle for 9 weeks. During the experimental period, all animals were fed a standard laboratory chow diet or western type diet containing 20% protein and 21% fat. It was based on casein, corn starch, glucose, cocoa butter, cellulose, minerals, cholesterol, and a vitamin mix (Feedlab, Kyungki-do, South Korea). Mice were randomly assigned to one of the three groups. The three groups of diets with or without interventions were as follows: 1) regular chow diet group (normal control, NC), 2) western diet with shear stress induction group (WD/Cast), and 3) western diet with shear stress induction after Smad decoy ODN treatment group (WD/Cast/Smad).

Shear stress and decoy ODN treatment

To induce standardized changes of shear stress *in vivo*, we used a cast which imposes a fixed geometry on the carotid vessel wall, thereby causing gradual stenosis resulting in decreased blood flow upstream from the cast, as previously described in detail [4]. Briefly, the animals were anesthetized with isoflurane, and the anterior cervival triangles were accessed by a sagittal anterior neck incision. Both halves of the cast were placed around the right common carotid artery and fixed with a suture. After wounds were closed, the animals were allowed to recover.

Decoy ODN were injected into the tail vein at 2nd, 4th, 6th, and 8th week in the group 2 and group 3. The non-viral vector, Trans IT In vivo Gene Delivery System (Mirus, WI, USA), was used for the delivery of decoy ODN. At the end of each treatment period (9 weeks), the animals were sacrificed by cervical dislocation, and carotid artery was collected. All surgical and experimental procedures used in the present study were approved by the Institutional Review Board Committee at Daegu Catholic University Medical Center which conforms to the US National Institutes of Health guidelines for care and use of laboratory animals.

Synthesis of ring type decoy ODN and selection of target sequences

The following sequences of ODN were utilized (Consensus sequence is underlined): Smad decoy ODN: 5'-CAGTCTAGACACGTGATCACGTGT-CTAGACTG-3'. Considering the feasibility of a decoy ODN makeup, we designed a ring-type decoy ODN. These ODN were annealed for 8 hours, while temperature was decreased from 80°C to 25°C. The Smad decoy ODN was predicted to form a stem-loop structure. Following

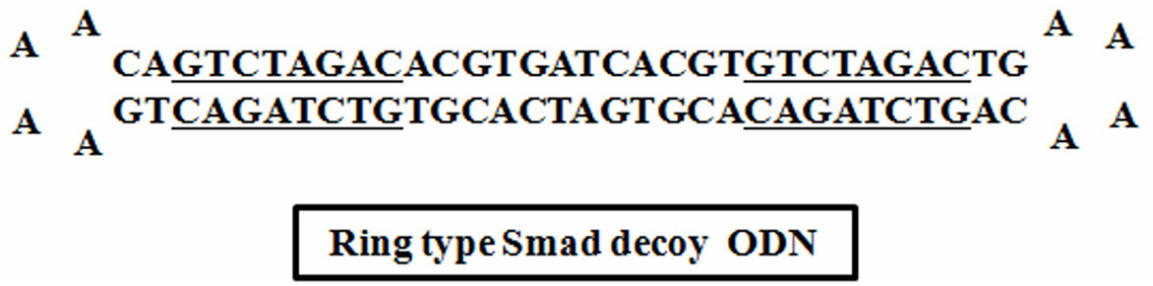

Figure 1. Structure of Smad decoy ODN. Binding sites of specific transcription factors are marked by underline.

the addition of T4 ligase (1U, Takara, Japan), the mixture was incubated for 18 hours at 16°C to generate a covalently ligated ring-type decoy molecule (**Figure 1**).

Histological analysis

All tissue specimens were fixed in 10% formalin for at least 24 hours at room temperature. After fixation, perpendicular sections to the anterior-posterior axis of the tissues were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Thin sections (4 μm) were mounted on glass slides, dewaxed, rehydrated to distilled water, and stained with Masson's trichrome and hematoxylin and eosin. Preparation of paraffin blocks followed, and cross-section taken from the blocks was stained with Masson's trichrome. As a part of the histological evaluation, all slides were examined by a pathologist, blinded to previous treatment, under a light microscope (Nikon, Tokyo, Japan).

Verhoeff's elastin staining

Paraffin-embedded sections (4 μm) were made and transferred to glass slides, and then were deparaffinized and dehydrated through following solutions: xylene 3 times for 3 minutes each, and ethanol serially for 3 minutes in each concentration of 100%, 95%, 80%, 75%, and 70%. Sections were then treated with Verhoeff' elastin staining solution (22 mL of alcoholic hematoxylin, 8 mL of 10% ferric chloride, and 8 mL of iodine solution) for 10 minutes, followed by washing in distilled water. Sections were briefly differentiated in 2% ferric chloride and were checked under the microscope. If differentiated excessively, staining-differentiation steps were repeated until proper staining pattern was obtained. Sections were then treated with 5% sodium thiosulfate for 1 minute, washed in tap water for 5 minutes, and counter-stained in van Gieson' staining solution (5 mL of 10% acid fuchsin, and 100 mL of saturated aqueous solution picric acid) for 1 minute. Sections were differentiated in 95% ethyl alcohol, 2 changes of absolute ethanol, 2 changes of xylene, and they were mounted on slide.

Immunohistochemical staining

Paraffin-embedded sections were deparaffinized with xylene, dehydrated in gradually decreasing concentrations of ethanol, and then treated with 3% hydrogen peroxidase in methanol for 10 minutes to block endogenous peroxidase activity. Tissue sections were immersed in 10 mM sodium citrate buffer (pH 6.0) for 5 minutes at 95°C. The last step was repeated using fresh 10 mM sodium citrate solution (pH 6.0). Sections were allowed to remain in the same solution while cooling for 20 minutes and were rinsed in PBS. Sections were incubated with primary antibody (1:100 dilution) for 1 hour at 37°C. Such primary antibodies as anti-TGF-β1 (R&D System, MN, USA), anti-PAI-1 (Santa Cruz, CA, USA), and α-SMA (Sigma, MO, USA) were used. Signal was visualized using an Envision system (DAKO, CA, USA) for 30 minutes at 37°C. DAB (3, 3'-diaminobenzidine tetrahydrochloride) was used as the coloring reagent, and hematoxylin was used as a counter stain.

Results

Western diet with shear stress led to a development of atherosclerotic lesions, and Smad decoy ODN suppressed the histological changes in atherosclerotic ApoE-/-mouse

We first examined the effect of western diet and cast placement on the development of atherosclerosis. Carotid artery was harvested at

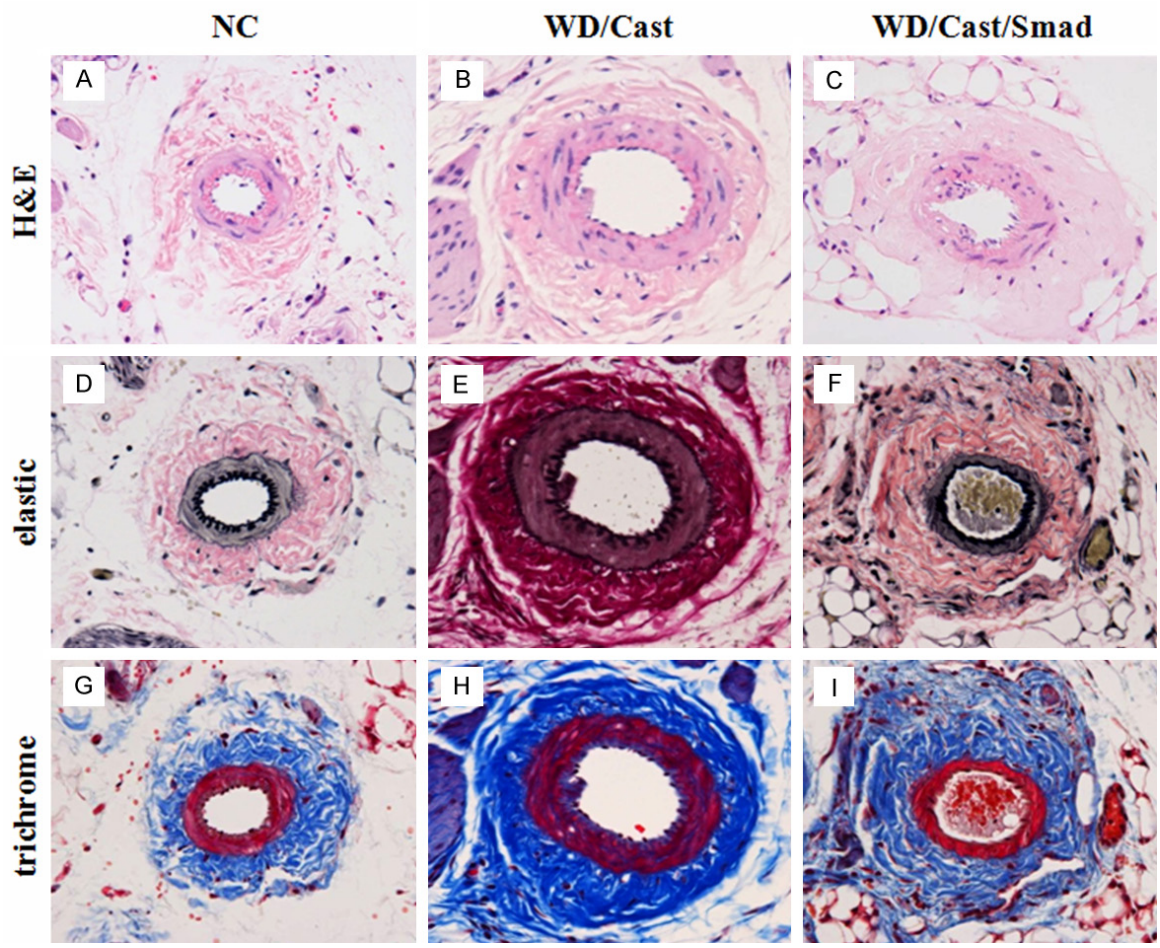

Figure 2. Smad decoy ODN suppressed the histological changes in atherosclerotic ApoE-/-mouse. A-C. Histological analyses of carotid arteries 9weeks after cast placement in ApoE-/-mice fed a western diet. D-F. Carotid sections are stain with Verhoeff's elastin which accentuates elastin fibers. G-I. Collagen fibers visualized by Masson's trichrome staining. Representative images from each study group. NC: normal control, WD/Cast: western diet with shear stress induction group, WD/Cast/Smad: western diet with shear stress induction after Smad decoy ODN treatment group. Magnification × 400.

the sacrifice to observe histological changes. H&E stained sections were examined to understand the extent and distribution of plaque formation as a determination of atherosclerotic lesions in ApoE-/-mouse. WD/Cast group showed increased plaque formation, thickened arterial walls, and intimal size compared with NC group (**Figure 2A** and **2B**). Structure of elastic tissue in arterial atherosclerotic lesions was seriously fragmented and disrupted, disorganized elastin fibers were observed in mice from WD/Cast group (**Figure 2E**). Also, collagen fibers visualized by trichrome staining were shown to be distinctly deposited in the WD/Cast group compared to NC group (**Figure 2H**). These results showed that western diet with shear stress (cast placement) led to the development of atherosclerotic lesions in ApoE-/-mouse.

Afterward, we investigated the effect of Smad decoy ODN on western diet with shear stress induced ApoE-/-mouse. Our study found that Smad decoy ODN treatment group (WD/Cast/Smad) showed decrease in the intima-media thickness and atherosclerotic lesions of the artery, compared to the WD/Cast group (**Figure 2C** and **2F**). Importantly, the intimal size and extent of arterial walls were attenuated and the collagen deposition was decreased by Smad decoy ODN treatment in the WD/Cast/Smad group (**Figure 2I**). Therefore, histological examination suggested that Smad decoy ODN prevented pathologic changes in western diet with

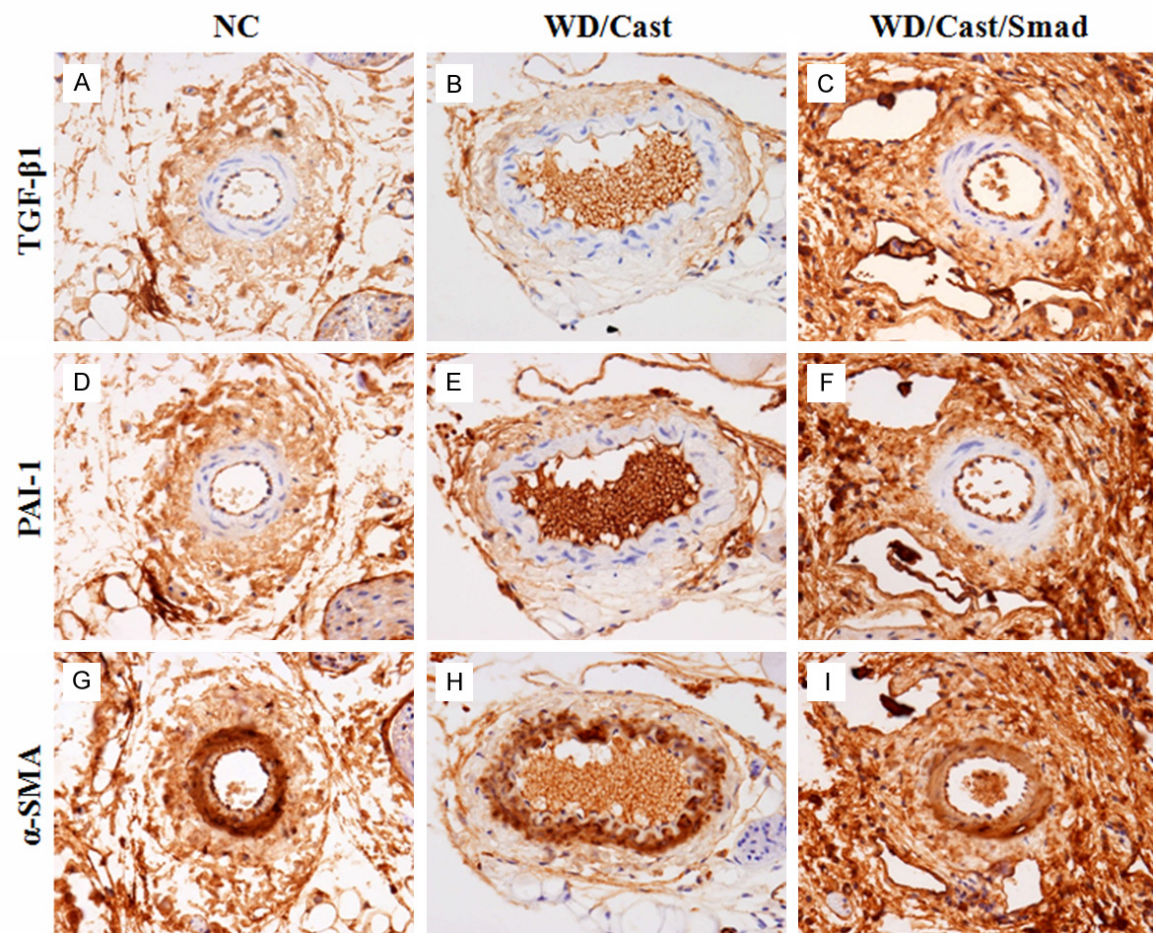

Figure 3. Smad decoy ODN prevented the extracellular matrix deposition in atherosclerotic ApoE-/-mouse. A-C. Representative macrographs show immunohistochemical staining for TGF-β1 in the carotid at 9weeks after cast placement. D-F. Immunohistochemical staining shows that Smad decoy suppresses the expression of PAI-1 in atherosclerotic ApoE-/-mouse. G-I. Immunohistochemical staining was used to evaluate the extent of α-SMA. Representative images from each study group. NC: normal control, WD/Cast: western diet with shear stress induction group, WD/Cast/Smad: western diet with shear stress induction after Smad decoy ODN treatment group. Magnification × 400.

shear stress-induced atherosclerotic Apo-E-/-mouse.

Smad decoy ODN prevented the extracellular matrix deposition in western diet with shear stress-induced atherosclerotic ApoE-/-mouse

Immunohistochemical stains were performed to evaluate the impact of Smad decoy ODN in the regulation of expression of the genes relevant to atherosclerosis, including TGF-β1, PAI-1, and α-SMA. TGF-β1 expression is up-regulated in plaque development and is known to promote atherosclerosis under a variety of circumstances, such as extracellular matrix (ECM) remodeling [25]. Also, PAI-1, a physiological regulator of plasminogen activation, is a representative profibrogenic gene, and its expression is transcriptionally regulated by the TGF-β1/Smad pathway [26]. As shown in **Figure 3A** and **3D**, the artery from NC group showed little expression of TGF-β1 and protease inhibitor such as PAI-1. However, WD/Cast group revealed markedly enhanced neointimal thicking, and these atherosclerosis markers were dramatically increased in WD/Cast group (**Figure 3B** and **3E**). Also, immunohistochemical examination showed that both neointimal and medial cells were positive for smooth muscle cell (SMC) marker such as α-SMA in WD/Cast (**Figure 3H**). However, administration of Smad decoy ODN showed down regulation of these proteins in WD/Cast/Smad group (**Figure 3C**, **3F** and **3I**). Therefore, immunohistochemical

stains showed that Smad decoy ODN treatment attenuated the expression of TGF-β1 and PAI-1. Also, smooth muscle cell activations were inhibited by Smad decoy ODN. These results suggest that Smad decoy ODN prevented the progression of atherosclerosis in western diet with shear stress-induced ApoE-/-mouse.

Discussion

Shear stress controls the expression of a number of genes involved in the endothelial cell functions, including TGF-β1, platelet–derived growth factor (PDGF), and tissue plasminogen activator. In particular, TGF-β1 is a factor that is elevated by shear stress [27-29]. TGF-β1 transmits its signal through type I and II serin/threonine kinase receptors and phosphorylates downstream targets Smad2 and Smad3. Subsequently, Smad2 and Smad3 interact with Smad4, translocate to the nucleus, and activate TGF-β1-responsive genes [30]. TGF-β1 ligands, its receptors, as well as Smad proteins have been found to be expressed in fibro-fatty lesions and fibrous plaques [20, 21]. Consistent with such an expression profile, TGF-β1 has been found to affect the properties and function of all cell types that are known to be present in atherosclerotic lesions, such as SMCs, monocyte/macrophage, and T cells [31]. Thus, strategies aimed at disrupting TGF-β1 production and/or blocking signal transduction with Smad has important theoretical and practical implications for producing the effective treatments for atherosclerosis.

The present study is the attempt to elucidate the effect of Smad decoy ODN in the western diet with shear stress-induced atherosclerotic ApoE-/-mouse. First, we examined the effect of western diet and cast placement on the development of atherosclerosis. A previous study reported that placement of the cast creates decreased shear stress upstream from the cast, increased shear stress in the cast, and oscillatory shear stress downstream from the cast [2]. Furthermore, several papers reported that high-fat or a high-cholesterol diet led to a development of atherosclerosis [32, 33]. Our results showed that western diet with cast placement induced increase in plaque formation, thickened arterial walls and intimal size. Also, collagen fibers were increased in western diet with cast placement induced ApoE-/-mouse. These results demonstrate that western diet with cast placement may effectively induce the development of atherosclerotic lesions in ApoE-/-mouse. Subsequently, we investigated the effects of Smad decoy ODN on western diet with cast placement-induced atherosclerotic ApoE-/-mouse. We previously reported that NF-κB and Sp1 chimeric decoy ODN efficiently suppressed the expression of specific genes in the animal model of atherosclerosis [34]. In addition, several experimental studies have shown that decoy therapy attenuates atherosclerosis [24, 35]. These studies are informative but it is yet to be demonstrated that Smad decoy ODN can prevent the development of atherosclerosis in the western diet with shear stress-induced ApoE-/-mouse model. *Grainger et al* showed that transgenic expression of apoliprotein promoted SMC proliferation and subsequent development of early vascular lesions by inhibiting proteolytic activation of TGF-β1 [36]. In additions, overexpression of TGF-β1 caused arterial intimal thickening largely consisted of increased ECM [37]. In the present study, our result showed that Smad decoy ODN suppressed atherosclerosis related genes such as TGF-β1, PAI-1 and α-SMA in western diet with shear stress induced atherosclerotic ApoE-/-mouse. Also, Smad decoy ODN treatment effectively inhibited the pathologic changes of atherosclerosis. These results suggest that Smad decoy ODN prevented the progression of atherosclerosis in the ApoE-/-mouse induced by western diet and shear stress.

In conclusion, our findings demonstrate that western diet with cast placement developed atherosclerosis in ApoE-/-mouse. Also, administration of Smad decoy ODN decreased the expression of TGF-β1, PAI-1, and α-SMA. In particular, Smad decoy ODN exerts anti-atherosclerotic affect against western diet with shear stress induced atherosclerotic ApoE-/-mouse via inhibiting the pathologic changes. These results demonstrate the therapeutic potential of Smad decoy ODN for the prevention of atherosclerosis induced in ApoE-/-mouse model by western diet with shear stress.

Acknowledgements

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (grant number 2014R1A1A2057-298).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Sung Won Youn, Department of Radiology, School of Medicine, Catholic University of Daegu, 3056-6, Daemyung-4-Dong, Nam-gu, Daegu, 705-718, South Korea. Tel: (+82) 53-650-4076; Fax: (+82) 53-650-4834; E-mail: ysw10adest@cu.ac.kr

References

[1] Civeira F. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis 2004; 173: 55-68.

[2] Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D and Bonaa KH. Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arterioscler Thromb Vasc Biol 1996; 16: 984-991.

[3] Warboys CM, Amini N, de Luca A and Evans PC. The role of blood flow in determining the sites of atherosclerotic plaques. F1000 Med Rep 2011; 3: 5.

[4] Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R and de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. Circulation 2006; 113: 2744-2753.

[5] Williams H, Johnson JL, Carson KG and Jackson CL. Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol 2002; 22: 788-792.

[6] Kamiya A and Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. Am J Physiol 1980; 239: H14-21.

[7] Kubis N, Checoury A, Tedgui A and Levy BI. Adaptive common carotid arteries remodeling after unilateral internal carotid artery occlusion in adult patients. Cardiovasc Res 2001; 50: 597-602.

[8] Wentzel JJ, Kloet J, Andhyiswara I, Oomen JA, Schuurbiers JC, de Smet BJ, Post MJ, de Kleijn D, Pasterkamp G, Borst C, Slager CJ and Krams R. Shear-stress and wall-stress regulation of vascular remodeling after balloon angioplasty: effect of matrix metalloproteinase inhibition. Circulation 2001; 104: 91-96.

[9] Pedersen EM, Oyre S, Agerbaek M, Kristensen IB, Ringgaard S, Boesiger P and Paaske WP. Distribution of early atherosclerotic lesions in the human abdominal aorta correlates with wall shear stresses measured in vivo. Eur J Vasc Endovasc Surg 1999; 18: 328-333.

[10] Cheng C, de Crom R, van Haperen R, Helderman F, Mousavi Gourabi B, van Damme LC, Kirschbaum SW, Slager CJ, van der Steen AF and Krams R. The role of shear stress in atherosclerosis: action through gene expression and inflammation? Cell Biochem Biophys 2004; 41: 279-294.

[11] Malek AM, Alper SL and Izumo S. Hemodynamic shear stress and its role in atherosclerosis. JAMA 1999; 282: 2035-2042.

[12] Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999; 340: 115-126.

[13] Getz GS. Thematic review series: the immune system and atherogenesis. Immune function in atherogenesis. J Lipid Res 2005; 46: 1-10.

[14] Nikol S, Isner JM, Pickering JG, Kearney M, Leclerc G and Weir L. Expression of transforming growth factor-beta 1 is increased in human vascular restenosis lesions. J Clin Invest 1992; 90: 1582-1592.

[15] Westerhausen DR Jr, Hopkins WE and Billadello JJ. Multiple transforming growth factor-beta-inducible elements regulate expression of the plasminogen activator inhibitor type-1 gene in Hep G2 cells. J Biol Chem 1991; 266: 1092-1100.

[16] Ignotz RA and Massague J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 1986; 261: 4337-4345.

[17] Laiho M, Ronnstrand L, Heino J, Decaprio JA, Ludlow JW, Livingston DM and Massague J. Control of junB and extracellular matrix protein expression by transforming growth factor-beta 1 is independent of simian virus 40 T antigen-sensitive growth-sensitive growth-inhibitory events. Mol Cell Biol 1991; 11: 972-978.

[18] Schulick AH, Taylor AJ, Zuo W, Qiu CB, Dong G, Woodward RN, Agah R, Roberts AB, Virmani R and Dichek DA. Overexpression of transforming growth factor beta1 in arterial endothelium causes hyperplasia, apoptosis, and cartilaginous metaplasia. Proc Natl Acad Sci U S A 1998; 95: 6983-6988.

[19] Nabel EG, Shum L, Pompili VJ, Yang ZY, San H, Shu HB, Liptay S, Gold L, Gordon D, and Derynck R, et al. Direct transfer of transforming growth factor beta 1 gene into arteries stimulates fibrocellular hyperplasia. Proc Natl Acad Sci U S A 1993; 90: 10759-10763.

[20] Kalinina N, Agrotis A, Antropova Y, Ilyinskaya O, Smirnov V, Tararak E and Bobik A. Smad expression in human atherosclerotic lesions: evidence for impaired TGF-beta/Smad signaling in smooth muscle cells of fibrofatty lesions. Arterioscler Thromb Vasc Biol 2004; 24: 1391-1396.

[21] Bobik A, Agrotis A, Kanellakis P, Dilley R, Krushinsky A, Smirnov V, Tararak E, Condron M and Kostolias G. Distinct patterns of transforming growth factor-beta isoform and receptor expression in human atherosclerotic lesions. Colocalization implicates TGF-beta in fibrofatty lesion development. Circulation 1999; 99: 2883-2891.

[22] Stone PH, Coskun AU, Kinlay S, Clark ME, Sonka M, Wahle A, Ilegbusi OJ, Yeghiazarians Y, Popma JJ, Orav J, Kuntz RE and Feldman CL. Effect of endothelial shear stress on the progression of coronary artery disease, vascular remodeling, and in-stent restenosis in humans: in vivo 6-month follow-up study. Circulation 2003; 108: 438-444.

[23] Morishita R, Higaki J, Tomita N and Ogihara T. Application of transcription factor "decoy" strategy as means of gene therapy and study of gene expression in cardiovascular disease. Circ Res 1998; 82: 1023-1028.

[24] Miyake T, Aoki M, Osako MK, Shimamura M, Nakagami H and Morishita R. Systemic administration of ribbon-type decoy oligodeoxynucleotide against nuclear factor kappaB and ets prevents abdominal aortic aneurysm in rat model. Mol Ther 2011; 19: 181-187.

[25] Xu S, Liu AC and Gotlieb AI. Common pathogenic features of atherosclerosis and calcific aortic stenosis: role of transforming growth factor-beta. Cardiovasc Pathol 2010; 19: 236-247.

[26] Hua X, Miller ZA, Benchabane H, Wrana JL and Lodish HF. Synergism between transcription factors TFE3 and Smad3 in transforming growth factor-beta-induced transcription of the Smad7 gene. J Biol Chem 2000; 275: 33205-33208.

[27] Resnick N, Collins T, Atkinson W, Bonthron DT, Dewey CF Jr and Gimbron MA Jr. Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear-stress-responsive element. Proc Natl Acad Sci U S A 1993; 90: 7908.

[28] Chun TH, Itoh H, Ogawa Y, Tamura N, Takaya K, Igaki T, Yamashita J, Doi K, Inoue M, Masatsugu K, Korenaga R, Ando J and Nakao K. Shear stress augments expression of C-type natriuretic peptide and adrenomedullin. Hypertension 1997; 29: 1296-1302.

[29] Ohno M, Cooke JP, Dzau VJ and Gibbons GH. Fluid shear stress induces endothelial transforming growth factor beta-1 transcription and production. Modulation by potassium channel blockade. J Clin Invest 1995; 95: 1363-1369.

[30] Massague J. How cells read TGF-beta signals. Nat Rev Mol Cell Biol 2000; 1: 169-178.

[31] Grainger DJ. Transforming growth factor beta and atherosclerosis: so far, so good for the protective cytokine hypothesis. Arterioscler Thromb Vasc Biol 2004; 24: 399-404.

[32] Schreyer SA, Wilson DL and LeBoeuf RC. C57BL/6 mice fed high fat diets as models for diabetes-accelerated atherosclerosis. Atherosclerosis 1998; 136: 17-24.

[33] Johansson ME, Hagg U, Wikstrom J, Wickman A, Bergstrom G and Gan LM. Haemodynamically significant plaque formation and regional endothelial dysfunction in cholesterol-fed ApoE-/- mice. Clin Sci (Lond) 2005; 108: 531-538.

[34] Lee WR, Kim KH, An HJ, Park YY, Kim KS, Lee CK, Min BK and Park KK. Effects of chimeric decoy oligodeoxynucleotide in the regulation of transcription factors NF-kappaB and Sp1 in an animal model of atherosclerosis. Basic Clin Pharmacol Toxicol 2013; 112: 236-243.

[35] Kim SJ, Park JH, Kim KH, Lee WR, Lee S, Kwon OC, Kim KS and Park KK. Effect of NF-kappaB decoy oligodeoxynucleotide on LPS/high-fat diet-induced atherosclerosis in an animal model. Basic Clin Pharmacol Toxicol 2010; 107: 925-930.

[36] Grainger DJ, Kemp PR, Liu AC, Lawn RM and Metcalfe JC. Activation of transforming growth factor-beta is inhibited in transgenic apolipoprotein(a) mice. Nature 1994; 370: 460-462.

[37] Kanzaki T, Tamura K, Takahashi K, Saito Y, Akikusa B, Oohashi H, Kasayuki N, Ueda M and Morisaki N. In vivo effect of TGF-beta 1. Enhanced intimal thickening by administration of TGF-beta 1 in rabbit arteries injured with a balloon catheter. Arterioscler Thromb Vasc Biol 1995; 15: 1951-1957.